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<td>Development of soy-based foods of high nutritive value for use in the Philippines; semi-annual scientific progress report, July-Dec. 1969</td>
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<th>12. DESCRIPTORS</th>
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<tr>
<td>(101) Cornell Univ. Dept. of Food Science and Technology</td>
<td>Philippines</td>
<td>CSD-1815 Res.</td>
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<td></td>
<td>Soybean</td>
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<th>14. CONTRACT NUMBER</th>
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<tr>
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<td>AID 590-1 (4-74)</td>
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Geneva, New York
December 31, 1969

TITLE: Development of Soy-Based Foods of High Nutritive Value for Use in the Philippines

FIFTH SEMI-ANNUAL SCIENTIFIC PROGRESS REPORT

FOR THE PERIOD: July 1 to December 31, 1969

CONTRACT NO.: AID/csd-1815

ISSUING OFFICE: Contracting Officer, Office of Procurement, Contract Service Division, Washington, D. C. 20523, ATTN: Administrator, Contract AID/csd-1815


EFFECTIVE DATE: June 30, 1967

CONTRACTOR: Cornell University, Ithaca, New York

PROJECT DIRECTOR: Willard B. Robinson, Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York 14456

ASSOC. PROJECT DIRECTOR: Keith H. Steinkraus, U.P.-College of Agriculture, Cornell-Ford Graduate Education Program, College, Laguna, Philippines
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I. Progress at Geneva

A. Engineering Studies

1. The use of a Comitrol for grinding soaked soybeans

The use of a Comitrol was tested in the Urschel Laboratories as a means of disintegrating soaked soybeans with water to form soy milk. The Comitrol is a high speed cutting mill designed to reduce foods to particles having a narrow range in size distribution. The food is fed onto the center of a revolving disc having radial impellers that throw it at a high velocity against stationary knives or blades. When knives are used, very thin slices can be uniformly obtained from small products. Replacement of the knives by blades, having sharp square corners, will yield a finely cut or homogenized material. Homogenization is achieved by the alternate compression and decompression of the particles as they pass successive blades.

- CUT PRODUCT
- BLADES
- REVOLVING IMPELLER
- PRODUCT
- DEPTH OF CUT
- REVOLVING IMPELLER
- SHARP CUTTING EDGES
- PRODUCT
- EXIT OF PRODUCT
- LIQUID
- SOLID PORTION RELEASED FROM COMPRESSION
- SOLID PORTION UNDER IMPACT AND COMPRESSION

Apart from the type of cutting edge used, the main variables in the operation of a Comitrol are:

(a) rotor speed - which may range from 3,600 to 36,000 rpm
(b) clearance between the impellers and the stationary blades
(c) distance between successive stationary blades
(d) temperature of the product - (hot materials cut better)

The rate at which a Comitrol is fed or, the pressure under which
the product is introduced to the cutting chamber, have little effect
on cutting action.

In the limited time that was available for these tests, three
experimental conditions were selected by Urschel personnel as being
most appropriate for disintegrating soaked soybeans based on past
experience with a variety of foods. The test conditions were as
follows:

<table>
<thead>
<tr>
<th>Test Condition</th>
<th>Test #1</th>
<th>Test #2</th>
<th>Test #3</th>
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<tr>
<td>Type of Head</td>
<td>Microcut</td>
<td>Microcut</td>
<td>Homogenizing</td>
</tr>
<tr>
<td>Head No.</td>
<td>100156</td>
<td>100084</td>
<td>222084</td>
</tr>
<tr>
<td>Rotor Speed (rpm)</td>
<td>9,450</td>
<td>9,450</td>
<td>9,450</td>
</tr>
<tr>
<td>Space Between Knives (in.)</td>
<td>0.0327</td>
<td>0.0207</td>
<td>0.007</td>
</tr>
<tr>
<td>Depth of Cut (in.)</td>
<td>0.059</td>
<td>0.018</td>
<td>0.007</td>
</tr>
<tr>
<td>Feed Material</td>
<td>15 lbs. soaked beans +</td>
<td>Slurry from</td>
<td>Test #2</td>
</tr>
<tr>
<td>Feed Time (min.)</td>
<td>1</td>
<td>1</td>
<td>0.3</td>
</tr>
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</table>

The soybeans were obtained on the local market and soaked for
2 hours in water at 122°F (50°C). The soak water was discarded and
fresh water at near the boiling point was added simultaneously with
the beans to the Comitrol. In order to achieve a reasonably uniform
feed to the Comitrol, each lot of beans and water was divided into
3 equal portions of 5 lbs. of beans and 15 lbs. of water and the
rate of their addition was gauged so that it took 20 seconds to add
a portion of beans and water to the Comitrol. To achieve the finest
particle subdivision, a slurry from a preliminary grinding (Test #2)
was passed through the Comitrol when a homogenizing head had been
substituted for the micro head.

Samples of the 3 slurries were analyzed for their particle size
distribution at Geneva using a wet sieving procedure employing
screens having mesh openings of 800, 400, 250, 160, 100, 70 and 45
microns. The solids remaining on the screens were dried and the
cumulative percentages of dried solids have been plotted in Figure 1
against the size of the screen mesh in microns.

The smaller knife spacing used in Test #2 gave a slightly finer
grind and there was no indication of plugging of the spaces between
the knives of the micro head. It was the opinion of Urschel personnel
that Head No. 180084 used in Test #2 might be used satisfactorily
when grinding a bean-water mixture having a ratio as low as 1 part
of beans to 2 parts of water.
The homogenizing head further reduced the size of the bean particles as shown in Figure 1. However, during the short duration of this test, it became apparent that the machine would soon be inoperative due to plugging of the head. Apparently, the centrifugal force imparted by the impellers caused a rapid separation of the particles and the larger and heavier material was thrown against the blades faster than they could be sheared. This resulted in a heavy deposition of solids across the face of the blades. If the coarser particles had been removed in a previous filtration or centrifugation step for recycling through the preliminary grinder, the homogenizing head might have been more effective.

In Figure 1 the results of particle size analyses are also presented from previous tests for purposes of comparison. In the earlier study, soybeans and water were ground in a Rietz Disintegrator and then passed through a Morehouse colloid mill fitted with carborundum plates. The Comitrol equipped with a Microcut head gave

Fig. 1. Size of particles in soy milks ground in various units.
a finer grind than the Disintegrator during the preliminary comminution of the beans. There was little difference in the degree of size reduction accomplished by the Comitrol having a homogenizing head and the colloid mill.

Summary

Preliminary tests were conducted at the Urschel Laboratories, Valparaiso, Indiana, on the use of their Comitrol for the preliminary grinding of a relatively thick mixture of soybeans and water (1:3) and further size reduction of the particles in the resulting slurry. Particle size analyses were made of the slurries in this laboratory by a wet sieving procedure.

On the basis of the screening analyses, the Comitrol equipped with a Microcut head is superior to a hammer type mill, such as the Rietz Disintegrator for the initial grinding of the beans. A Comitrol having a homogenizing head gave approximately the same degree of size reduction as a stone mill.

B. Biochemical Studies

1. Enzymatic Removal of Soy Milk Oligosaccharides

One of the qualitative problems in soy milk is its tendency to induce flatulence. Recently, it has been suggested by some investigators that the flatulence caused by soy products could be due, at least in part, to their relatively high content of galactose-containing oligosaccharides, especially stachyose and raffinose.

In earlier studies, it was found that considerable numbers of fungal strains belonging to the genus Aspergillus exhibited pronounced abilities to produce galacto-oligosaccharide-decomposing enzymes, α-galactosidases (E.C.3.2.1.22).

On the basis of these factors, a method for enzymatic removal of galacto-oligosaccharides from soy milk by a partially purified enzyme preparation from a commercial acid-protease product of A. saitoi was investigated.

(a) Partial Purification of the Enzyme

Contamination of the enzyme preparation with protease activity must be strictly avoided, because it may cause many undesirable effects on soy milk such as coagulation, precipitation of protein, formation of bitterness, etc. Fortunately, the α-galactosidase from A. saitoi has an especially high molecular weight (c.a. 290,000), hence the separation of it from protease(s)
could be attained by means of a membrane filtration method. From another point of view, this fact indicates the possibility that the enzyme can be obtained economically as a by-product of purified protease production.

(b) Characteristics of the Enzyme

The α-galactosidase from A. saitoi soy milk has been found to have other advantageous characteristics. Since the optimum pH for the enzyme activity is in the natural pH range of soy milk, there is no need to change the original pH value of soy milk. In addition, the enzyme itself is very stable in the same pH range.

Further, the activity of the enzyme is relatively insensitive to the presence of an end product, α-D-galactose, in the reaction mixture.

(c) Enzymatic Hydrolysis of Soy Milk Oligosaccharides

Thin layer chromatography indicated that the addition of small amounts of the partially purified α-galactosidase preparation to soy milk followed by incubation for a few hours at 50°C, resulted in the complete hydrolysis of both stachyose and raffinose. In this case, no undesirable qualitative change in soy milk occurred. On the contrary, as the result of hydrolysis of the oligosaccharides, the organoleptic sweetness of soy milk increased significantly.

The method presented promises to be an economical and convenient procedure for the removal of oligosaccharides from soy milk.

2. Pyroglutamic acid

Recently some Japanese workers reported the isolation of a bitter peptide thought to contain pyroglutamic acid (PCA). Since PCA may contribute to organoleptic properties of soybean products, investigations have been undertaken to determine its presence in soy milk. Thus far, no detectable quantity of PCA in either a control or oven-heated soy milk has been found. It is possible that the extraction procedure was not appropriate for splitting PCA from a soy peptide, however, the procedure used was adequate for extracting PCA from beet puree.

3. Dipeptide studies

Other research is under way to determine the dipeptides present in soy milk since they may be responsible for the bitter, astringent taste. Three large samples of soy milk have been prepared in the pilot plant for use in flavor studies, as well as chemical studies, on the possible role of peptides in soy flavor.
Thus far, elution times and color ratios of 39 dipeptides have been determined by automated column chromatography procedures. Subsequent analysis of six picric acid supernatants revealed four peaks of significant size which were eluted in the dipeptide range. It appears that the quantity of each of these eluted compounds is dependent upon the processing procedures used in the preparation of soy milk. Published information from other laboratories has shown the presence of two dipeptides in soybeans. Both of these previously isolated dipeptides have a sour to astringent flavor. No mention has been made in the literature concerning the possibility of other dipeptides. If these compounds and the processes by which they are released can be identified, we may be able to improve the flavor of soy milk.

C. Nutritional Studies

1. Protein Quality of Roasted Soybeans

Amino acid analyses on dry roasted soybeans have been completed. Results indicate that roasting affects the total and available lysine, cystine, and tryptophan content, although not consistently. Generally speaking, the heat-susceptible amino acids decrease as the degree of roast increases. The roasting process improved the PER value from an average value of 0.61 for raw beans to 1.48 for roasted soybeans. Roasting provides a good method for the utilization of whole soybeans as a processed food in areas of the world with a minimum of technology.

2. Methionine supplementation of alkali-treated soy milk

Amino acid studies of soy milk have indicated that cystine decreases in milk prepared with an alkali soak. Therefore, we have investigated the effect of supplementing alkali-treated soy milk with methionine. The PER value of pH 9.0 soy milk was improved from 1.47 to 2.82 by methionine supplementation, while the PER value of pH 6.5 soy milk was improved from 2.22 to 2.86 by the addition of methionine. The results clearly indicate that a PER value of 2.82 to 2.86 is maximum for methionine-supplemented soy milk that was prepared by an alkali-soak procedure.

II. Progress at Los Baños

A. Pilot Plant Operations

The temporary pilot plant is now in full operation. All units of equipment are being used regularly. All soy milk is now made in the pilot plant. Quality control procedures have been established in order to ensure that a good quality milk can always be made under highly reproducible conditions.
A Taiwan-made, motorized carborundum-stone grinder was purchased for about $300 (approximately one twelfth the cost of the Rietz disintegrator). This is the type of grinder that is often used by small soy milk and soy curd factories. Our experiments with this grinder show that it is suitable for the boiling-water grinding of soybeans giving a milk that is free from the beany flavor. In taste tests with an experienced panel, milk made in the Rietz disintegrator scored a mean of 7.8 on a nine-point scale while milk made in the stone grinder scored 7.7. The grinder has a greater capacity than the Rietz disintegrator.

A hand-operated stone grinder of the type that is used for grinding rice was used for grinding soybeans but its production capacity was only 200 ml of milk per hour. A hand-operated stone grinder of a modified design was made especially to order at a cost of 20 pesos (five dollars). This modified grinder has a production capacity of 5 to 10 liters per hour. This stone grinder has proved suitable for the boiling-water grinding technique, giving a milk that is free from the beany flavor. However, the milk has a strong chalky flavor that is objectionable. We plan to continue research with this type of grinder in order to diminish the chalky flavor and to further increase the production capacity.

B. Soy Milk

1. Cost of beverage

It is important that every effort be made to reduce the cost of the soy milk by every available means in order to bring the cost of a commercial product within reach of as much of the population as possible. Vanilla essence can be added at a cost of only one cent per 10 gallons.

2. Chocolate flavor

Experiments have been initiated with chocolate-flavored soy milk using a commercial supply of cocoa powder and cocoa flavoring with a view to making the chocolate-flavored soy milk at lower cost.

3. Effect of added oil

Regular soy milk was homogenized with 1%, 2%, and 3% of added coconut oil. The homogenized oil imparted a whiter appearance to the milk but it did not cover the graying that develops on standing. The taste panel showed a slight preference for the milk with added oil. Some panel members said it made the milk taste more creamy.
4. Alkali soak

Milk was made with beans soaked in water and in 0.1%, 0.2%, 0.3% sodium hydroxide solutions respectively. The taste panel consistently preferred the milk made from beans that were given no alkali treatment. They complained of a "soapy" taste in all alkali treated samples and maintained this opinion through several series of experiments. This result is the opposite of the results found by the taste panel in Geneva. In view of this difference, the use of alkali treatments will be given further study at both locations.

5. Storage study

Two hundred bottles of soy milk made in August 1969 were put aside for a storage study under ambient tropical conditions. After 1/2 month of storage, there is almost no deterioration of flavor, very little increase in sediment, no precipitation of the protein, and a slight darkening of color. In fact, the flavor seems to become smoother and slightly more acceptable after one month of storage.

6. Packaging and labeling

Five gross of unbranded, plain, 7-oz. bottles were purchased for soy milk containers. This supply is replenished by new purchases of unbranded bottles whenever necessary. The unbranded bottles make it easier to detect visual changes in the milk, and they make a more presentable item to be shown to visitors than do branded bottles.

An inexpensive plain label has been made by typing a stencil and running copies off on a mimeograph machine. Most of the bottled milk is now labeled in order to make the product more presentable and to inform people about the nature of the beverage. The label bears the following inscription:

PHILSOY

a nutritious protein drink

Ingredients:
Philippine soybeans 10%
Philippine sugar 9%
Imported flavoring 0.003%
Made with Los Baños Spring Water

Food Technology Pilot Plant
U. P. College of Agriculture
College, Laguna, Philippines
C. Other Soy Products

1. A supply of dried tempeh culture (Rhizopus oligosporus) has been built up in readiness for demonstrations of tempeh production.

2. Miss V. Castro is working on the development of fermented cheeses from soy milk and mixtures of soy milk and cows milk for her M. S. degree. Some pleasant cheese-like flavors are being obtained, but soft texture of the cheeses is a major problem.

D. Extension Activities

1. Philippine extension

Samples of the standard milk made in the pilot plant under strict quality control have been taken to a number of people in government and in industry. A verbal explanation of the aims of the research contract was given to each person visited. The objective was to make them familiar with the new improved flavor of soy milk, and to arouse interest in starting up a commercial soy milk operation that would sell soy milk through regular retail channels. The following people have been visited:

Dr. Engel, Dr. Keeve, Dr. Breitenback, Mr. Ruppert, and Mr. Gelbard of AID Manila.

Mrs. Pesigan, Director, Food and Drug Administration.

Mr. A. Lao, General Manager, Foodmasters, Inc.

Dr. I. Pablo, Director, Philippine Institute of Nutrition, Food Science and Technology.

Dr. K. V. Bailey, W. H. O., Manila.

Dr. Jiminez, Food Factory Manager, Philippine Union College.

Mrs. C. Llavore, Asst. Chief, Home Economics Division, Bureau of Public Schools.

Mrs. Gonzales, Head, Food Research Section, National Institute of Science and Technology.

Dr. C. Dayrit, Research Director, United Drug Company.

Mr. J. de Jesus, Executive Secretary Philippine Soybean Producers Association.

Mr. T. Henares, President, Alakor Corporation.
Mr. A. Cuyagan, General Manager, San Miguel Glass Plant.

Dr. Zialcita, Research Director, San Miguel Corporation.

Dr. B. Inciong, Executive Director, Nutrition Foundation of the Philippines.

Dr. C. Pascual, Director, Food and Nutrition Research Center, National Science Development Board.

Mr. T. Westphal, Marketing Manager, Philippine Packing Corporation (Del Monte).

Dr. L. Sumabat, Deputy Director, National Nutrition Program, Department of Public Health.

Mr. G. Evarista, Head, R & D, Ovaltine Product, Philippines.

A number of visitors to Los Baños have also been exposed to soy milk and told about our program. This includes about 30 students with Dr. Bradfield at IRRI, 17 agricultural scientists from India and Indonesia, Mr. C. Murray, Advisor to Bangpra Agricultural College, Thailand and a number of others.

Almost all of these people had a very favorable attitude towards soy milk and its potential for commercial development in the Philippines. With very few exceptions, they were enthusiastic about the improved acceptability due to the absence of the beany flavor.

A complete demonstration of soy milk manufacture in the pilot plant has been given to representatives of Food Masters, Inc. and AID representatives from Manila. We expect to be giving demonstrations and discussing the feasibility of commercial scale operations with other industry representatives in the near future.

2. AID India and Southeast Asia

Cooperation with the University of Illinois-U. S. AID soybean project at the U. P. Agricultural University, Pant Nagar, India has continued. Additional demonstrations of soybean processing and soybean technology were made to Indian women, both housewives and home demonstration agents, at Pant Nagar in the period June–July, 1969. They were taught how to make and utilize soybean milk, soybean curd, soybean cotyledons, soybean residue, Indonesian tempeh, and soybean nuts. The procedure used was to demonstrate the production of these various products in the morning sessions and then invite the participants to return for an afternoon session during which time they actually prepared the products. The Indian women displayed remarkable ingenuity in adapting the various soybean products to
Indian taste using varieties of spices and flavorings available in India. Soybean milk was flavored with almond extract and cardamon. The resulting soy milk was lacking the usual soy milk flavor and appeared to be highly acceptable to the Indian women. The soybean residue was processed by heating it with ghee and added sugar to make a confection. Soybean cotyledons were incorporated in a number of tasty Indian dishes. The net result was that the University of Illinois project hired a number of the Indian women to work on recipes. A book will be published within the next year on the use of soybeans in Indian cooking.

Cornell University has been extending help to the University of Illinois project and the U. P. Agricultural University working in conjunction with engineers (Servotech-Bombay) designing such a factory for solvent extraction and deflavoring of the soybean base and subsequent production of the "milk". The engineering firm is contributing their services at cost in a humanitarian contribution toward improving nutrition particularly for infants and children in India. As this project develops over the next year or so, it is hoped that the project will involve the active participation and collaboration of all three Universities - Cornell, University of Illinois, and U. P. Agricultural University, Pant Nagar. To achieve the objective of an imitation milk as similar to cow's milk as good quality margarine is to butter is a difficult project. However, with active collaboration of scientists in these three universities, along with the support of U. S. AID, it is believed that the objective can be reached.
III. Publications

A. Published


B. In Process


Fukushima, D., and J. P. Van Buren. 1970. Mechanisms of protein insolubilization during the drying of soy milk (Submitted to *Cereal Chemistry*).


Sugimoto, H., and J. P. Van Buren. 1970. Removal of oligosaccharides from soy milk by an enzyme from *Aspergillus saitoi*. (Submitted to *Journal of Food Science*).
